PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
JWJ01015WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/000218	21.01.2005	23.01.2004
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
LINGVITAE AS et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 12 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.11.2005	06.02.2006
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2005/000218

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-16 as originally filed

Claims, Numbers

1-33 as originally filed

Drawings, Sheets

1/4-4/4 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 27-32

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 27-32

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-26, 33 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2, 16-18, 20, 22-26
	No:	Claims	1, 3-15, 19, 21, 33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-26, 33
Industrial applicability (IA)	Yes:	Claims	1-26, 33
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1 DOCUMENTS

The following documents are referred to in this communication:

D1: WO 01/61037 A (Landegren, Ulf; Fredriksson, Simon) (2001-08-23)
D2: WO 03/031591 A (Superarray, INC) (2003-04-17)
D3: US-A-5 604 097 (Brenner et al) (1997-02-18)
D4: US 2002/028458 A1 (Lexow Preben) (2002-03-07)
D5: WO 01/00816 A (Complete Genomics AS; Lexow, Preben) (2001-01-04)
D6: DEAMER D W ET AL: "Nanopores and nucleic acids: prospects for ultrarapid sequencing" Trends in Biotechnology, vol. 18, no. 4, April 2000, pages 147-151, XP004194002
D7: BURGESS J K: "Gene expression studies using microarrays." Clinical and Experimental Pharmacology & Physiology. vol. 28, no. 4, April 2001, pages 321-328, XP002325541

2 Re Item IV
Lack of unity of invention

2.1 The following separate inventions or groups of inventions do not appear to be linked as to form a single general concept:

Invention 1: Claims 1 - 26, 33
Methods and kit to quantify or detect molecule(s) in a sample comprising the step of attaching a unique molecular tag to the molecules in the sample.

Invention 2 Claims 27 - 30
Method for determining the sequence of a polynucleotide in a sample comprising the step of attaching a unique molecular tag to polynucleotides in the sample.

Invention 3 Claims 31 - 32
Method for determining the sample origin of a biological molecule comprising the step

of attaching a molecular tag specific for the sample to the molecule in the sample.

2.2 The reasoning for the lack of unity of the present application within the meaning of Rule 13 PCT is as follows:

2.2.1 The problem to be solved by the present application appears to be the provision of methods comprising the step of rendering molecules identifiable.
The solution proposed in the present application are methods comprising the step of attaching a molecular tag to the molecules in the sample.
Hence, the common concept possibly linking inventions 1 - 3 appears to be the provision of methods comprising the step of attaching a molecular tag to the molecules in the sample.
However, such methods comprising the step of attaching a molecular tag to the molecules in the sample to render the molecules identifiable are already known in the prior art (cf. D1: Clm. 1; cf. D2: Clms. 1 - 4, 38; cf. D3: Clm. 21; cf. D4: Clms. 1-4; cf. D5: Clms. 16-25).
Therefore, the above defined common concept lacks novelty and cannot be considered as inventive. It thus does not represent a single general inventive concept as required by Rule 13.1 PCT: The present application merely provides 3 alternative solutions for the above defined problem.
Hence, the present application lacks unity (Rule 13 PCT).

2.2.2 No further features shared by the 3 inventions listed above could be identified, which would be considered as special technical features in the sense of Rule 13.2 PCT.
Therefore, the 3 inventions listed above are not unitary according to Rule 13 PCT.

2.3 As the Applicant has not had a search report drawn up on the other inventions, the application will be prosecuted on the basis of the invention in respect of which a search has already been carried out, in other words the invention first mentioned in the claims.

3 Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability

3.1 NOVELTY

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims **1, 3 - 15, 19, 21** and **33** is not new in the sense of Article 33(2) PCT:

3.1.1 The document D1 discloses:

3.1.1.1 A method for detecting and/or quantifying one or more analytes (polymeric molecules, proteins, antibodies, nucleic acids (cf. D1: Clm. 4; p. 3, par. 1)) in a sample by binding two or more proximity probes comprising a polynucleotide molecular tag of defined sequence (cf. for example D1: page 4, l. 1-3) to the analytes in the sample, carrying out the analysis procedure using the molecules in the sample, i.e. binding, ligating and/or polymerase amplifying the tags, and determining the number of molecules present in the sample on the basis of the tags (cf. D1: Clms. 1 - 7; Figs. 1-10; p. 2, par. 2-4; p. 3, par. 3 - p. 4, par. 3);

3.1.1.2 wherein the tag is identified in a read-out step in a manner ensuring that each tag is read at least once (cf. D1: Clm. 2);

3.1.1.3 wherein the tag comprises an antibody or aptamer (cf. D1: Clm. 3).

3.1.1.4 A method for detecting the presence of a molecule(s) in a sample comprising contacting the sample with two or more proximity probes having affinity for different parts of the target molecule, which comprise a polynucleotide molecular tag comprising a sequence that identifies the individual molecule and the class of target molecule (cf. D1: Example 4; Fig. 14: individual sequences Seq ID A and B and sequences specific for all proteins detected in the sample: primers B and F and universal ligation splint), and wherein on binding of that at least two proximity probes to the target molecule, two or more molecular tags are ligated and the ligated polynucleotide is detected (cf. D1: Clms. 1 - 7; Figs. 1-10; p. 2, par. 2-4).

3.1.1.5 A kit comprising a discrete compartment comprising one or more molecular tags as defined in any of claims 9 - 12 and 17 (cf. D1: Clm. 8).

The subject-matter of claims **1, 3 - 15, 19, 21** and **33** therefore appears not to be new (Art. 33(2) PCT) over D1.

3.1.2 The document D2 discloses:

3.1.2.1 A method for quantifying a plurality of different non-nucleic acid targets in a sample by providing a plurality of reporter ligands comprising a oligonucleotide identification tag of defined sequence, carrying out the analysis procedure using the molecules in the sample and determining the number of molecules present in the sample on the basis of the tags (cf. D2: Clm. 1; Clms. 22-25; page 7, par. 0018-0022: disclosure of so-called "oligonucleotide ID tags" with defined sequence);

3.1.2.2 wherein the reporter ligand/tag comprises an antibody or a polynucleotide derived from an in vitro evaluation (aptamer) (cf. D2: Clm. 20);

3.1.2.3 wherein the oligonucleotide ID tag is polymerase amplified (cf. D2: Clms. 30-33).

3.1.2.4 A kit comprising a discrete compartment comprising one or more molecular tags as defined in any of claims 9 - 12 and 17 (cf. D2: Clms. 40, 38).

The subject-matter of claims **1, 3 - 13, 33** therefore appears not to be new (Art. 33(2) PCT) over D2.

3.2 INVENTIVE STEP

3.2.1 The subject-matter of claims **1, 3 - 15, 19, 21** and **33** has been found not new in the sense of Article 33(2) PCT (see paragraph 2 above) and hence is also not considered as being inventive in the sense of Article 33(3) PCT.

3.2.2 Independent Claim **23**

3.2.2.1 The document D2, which is regarded as being the closest prior art to the subject-matter of claim **23**, discloses a method for detecting the presence of molecules present on the outer-surface of a cell or membrane comprising contacting the cell or membrane with a polynucleotide molecular tag of defined sequence and detecting the molecules on the basis of the tags (Clms. 1-4; Clms. 22-25), wherein the polynucleotide molecular tag comprise a nucleotide sequence that identifies the class of outer -surface molecule and the individual molecule (p. 68, par. 192 - p. 69, par 193; Fig. 8).

3.2.2.2 The subject-matter of claim **23** therefore differs from the teachings of D2 in that the

sample is contacted with two or more polynucleotide molecular tags having affinity for different parts of the target molecule, and in that on binding of that at least two polynucleotide molecular tags to the target molecule, the two or more molecular tags are ligated and the ligated polynucleotide is detected.

3.2.2.3 The technical effect of said difference appears to be a higher specificity of detection of molecules.

3.2.2.4 The problem to be solved by the present invention may therefore be regarded as the provision of an improved method to detect specific molecules present on the outer surface of a cell or membrane.

3.2.2.5 The solution proposed in claim **23** of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
The detection of molecules in solution using proximity-dependent DNA ligation assay and the advantages of such assays for the specificity for detection is already known in the prior art (cf. D1: Clms. 1 - 7; Figs. 1-10; p. 2, par. 2-4).
Therefore, the person skilled in the art would have applied the proximity-ligation technique known from D1 to a method for detection of outer-surface molecules from D1, thereby arriving at the method according to claim 23 of the present application without the use of inventive skills.
Hence, the subject-matter of claim **23** of the present application cannot be considered as inventive in the sense of Art. 33(3) PCT.

3.2.3 The additional subject-matter of dependent claims **2** and **17** is that the molecular tag has incorporated or is a sample identification portion. The additional subject-matter of dependent claims **22** and **24** is that the ligated polynucleotide molecular tag (as disclosed in D1: Clms. 1 - 7; Figs. 1-10; p. 2, par. 2-4) further comprises a sample identification portion.
The use of such sample identifier molecular tags for labelling the molecules from different samples with a different label to be able to distinguish them from each other in a later analysis procedure is well-known in the prior art, see for example D7, wherein the molecular tag is either the dye Cy3 or the dye Cy5, depending on the sample origin (cf. D7: Fig. 1 and page 325). Hence, the person skilled in the art would have combined a method to quantify or detect a molecule in a sample as disclosed in D1 or D2 with the use of the sample identification tags of D7, thereby arriving at a method of claims **2, 17, 22** and **24** of the present application without

the use of inventive skills.
Therefore, the subject-matter of dependent claims **2, 17, 22** and **24** cannot be considered as inventive (Art. 33(3) PCT) in view of the prior art documents D1, (D2) and D7.

3.2.4 Dependent claims **16, 18, 20, 25, 26** and **33** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect inventive step as they comprise merely routine procedures, which the person skilled in the art would choose, depending on the circumstances, in order to solve the problem posed without the use of inventive skills (see documents D2, D4, D5 and D6 and the corresponding passages cited in the search report).

In view of the above, the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims **1 - 26** and **33** does not involve an inventive step in the sense of Article 33(3) PCT.

4 Re Item VIII

Certain observations on the international application (Clarity (Article 6 PCT))

4.1 The subject-matter of claim **1** comprises a method comprising the steps of "attaching a unique molecular tag to substantially all of the molecules in the sample".

4.1.1 In said expression, the relative term "substantially all" has no well-recognised meaning and renders the definition of the subject-matter of said claim unclear (Article 6 PCT).

4.1.2 Furthermore, in said expression, it is not clear, whether the unique molecular tag is attached to substantially all individual molecules in the sample or to all molecules of the same type (e.g. mRNA with the same nucleic acid sequence or protein with the same amino acid sequence).
Fig. 1 of the present application appears to indicate that the intention of the Applicant is to attach such a unique tag to all individual molecules.
However, is not clear how such a tag could be attached to "substantially all of the molecules in the sample". It appears that the claim attempts to define the subject-

matter in terms of the result to be achieved, i.e. the labelling of substantially all molecules in the sample, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

Therefore, the subject-matter of claim **1** and further depending claims is unclear, Article 6 PCT.

For a preliminary examination of novelty and inventive step, the subject-matter of claim **1** has been interpreted in the broader sense that the unique molecular tag is attached to substantially all molecules of the same type since it appears that the description of the present application does not disclose how such a unique tag could be attached to all individual molecules.

4.2 The subject-matter of claim **4** comprises a method wherein "the read-out step is carried out in a manner that ensures that each tag in the original sample is read at least once".

It therefore appears that the claim attempts to define the subject-matter in terms of the result to be achieved, i.e. that each tag should be read at least once, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

Therefore, the subject-matter of claim **4** is unclear, Article 6 PCT.

4.3 The term "class of outer-surface molecule" used in claim **23** is vague and unclear and hence leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear, Article 6 PCT.